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ScienceDirect
Biochemical Systematics and Ecology 35 (2007) 489–500
biochemical systematics and ecology
www.elsevier.com/locate/biochemsyseco

Intraspecific variation of *Melaleuca quinquenervia* leaf oils in its naturalized range in Florida, the Caribbean, and Hawaii

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Received 21 December 2006; accepted 4 March 2007

Abstract

The invasive tree *Melaleuca quinquenervia* threatens the biodiversity of the Florida Everglades and adjacent areas. Biological control efforts have resulted in the release of three Australian insect species. Not all populations of the plant, however, are equally nutritious to the biological control agents. For example, previous results indicated that *Oxyops vitiosa* had reduced biomass and fecundity when fed different *M. quinquenervia* chemical variants. To find suitable sites for release of this herbivore species and locations where nursery sites can be developed for mass production of weevils, we studied the natural terpenoid variation in this plant throughout its range in Florida. For comparison, we also examined the terpenoid variation in naturalized populations in the Caribbean, Hawaii, and Australia. We found that two chemical variants or chemotypes exist in Florida, each dominated by one sesquiterpene, either *E*-nerolidol or viridiflorol. In the 28 populations studied in Florida no regional trends in chemotype dominance were found. More than half (16 of 28) of the populations were composed of between 34 and 66% of both chemotypes. A similar number of populations were dominated (>66%) by either the *E*-nerolidol (eight populations) or the viridiflorol (six populations) chemotype plants. Additionally, the total amount of terpenoids in leaves from plants grown north of the invasive range of *M. quinquenervia* was significantly reduced compared with invasive populations. The total amount of terpenoids in leaves from plants grown in Australia and the Caribbean was significantly greater than those in leaves from Florida and Hawaii. These results identified eight populations that would be most suitable for initial releases and where field nurseries could be established for on-site mass production.
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Keywords: Biological control; Chemotypes; Invasive species; *Melaleuca quinquenervia*; Naturalized populations; Terpenoids

1. Introduction

The environmental weed *Melaleuca quinquenervia* (Cav.) S.T. Blake (Myrtaceae) threatens the biodiversity of the south Florida ecosystem, including the Everglades National Park and adjacent natural areas (FLEPPC Plant List Committee, 2003). Currently, *M. quinquenervia* occupies more than 200,000 ha in south Florida and infests a variety of

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0305-1978/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.bse.2007.03.007

habitats (Bodle et al., 1994). This Australian species was introduced as early as 1886 and was planted widely in Florida as an ornamental and for erosion control (Turner et al., 1998; Dray et al., 2006). *M. quinquenervia* was also introduced to other locations throughout the US (e.g., California, Hawaii, and Texas), the Caribbean (e.g., Puerto Rico, The Bahamas, and the Virgin Islands), and Central American (e.g., Costa Rica). In many cases (California, Texas, the Virgin Islands, and Costa Rica) the plant did not naturalize, whereas in other areas (e.g., Florida, Hawaii, and the Caribbean), naturalized populations resulted from these introductions.

In many aromatic plant species, as in the family Myrtaceae (Brophy and Doran, 1996), different chemical variants are well-known and have been identified as distinct chemotypes. Examples of secondary compound variability or chemotypes in invasive weeds targeted for biological control include *Euphorbia esula* L. Euphorbiaceae (Holden and Mahlberg, 1992), *Hypericum perforatum* L. Clusiaceae (Sirvent et al., 2002), *Lantana camara* L. Verbenaceae (Randrianalijaona et al., 2005), and *Senecio jacobaea* L. Asteraceae (Macel et al., 2002). The variation in constituents studied from the leaves of *M. quinquenervia* in its native range (Ireland et al., 2002) and in Florida (Wheeler, 2006) indicates that at least two chemotypes exist within the species. Analyses of cultivated populations of this species from other regions of the world indicate the existence of numerous additional chemical variants not observed here (e.g., Trilles et al., 2006). One Florida chemotype is referred to here by its primary sesquiterpene *E*-nerolidol (chemotype I) and another by the sesquiterpene viridiflorol (chemotype II). The two Florida chemotypes are readily distinguished by the analysis of leaves with gas chromatography (GC) (Wheeler, 2006). However, only limited surveys have been conducted in the tree's adventive range, and possibly broader sampling will result in the discovery of additional chemotypes, trends in chemotype distributions regionally, and elucidate chemotype variation within *Melaleuca* stands.

Biological control activities in Florida against invasive populations of *M. quinquenervia* have resulted in the release of three insect species, the weevil *O. vitiosa* Pascoe (Center et al., 2000), a psyllid *Boreioglycaspis melaleucae* Moore (Center et al., 2006), and a gall fly *Fergusonina turneri* Taylor (Rayamajhi et al., 2002; Blackwood et al., 2006). All but the gall fly are widely established in Florida and impacting the host (Pratt et al., 2005a; Center et al., 2006; Franks et al., 2006). Both the weevil and the psyllid, however, are known to be sensitive to the variable levels of secondary metabolites of this weed. When fed the viridiflorol chemotype leaves, performance and fecundity are reduced in the weevil biological control agent *O. vitiosa* (Wheeler, 2006). Additionally, oviposition increases in the psyllid *B. melaleucae* when fed leaves of the viridiflorol chemotype (Wheeler and Ordung, 2005).

The purpose of this study was to characterize the foliar terpenoid profiles of different populations of *M. quinquenervia*. An extensive survey throughout the naturalized range of the plant will assist in the determination of the number of chemotypes that exist in Florida, Hawaii, and the Caribbean populations. These studies will identify both the most suitable release sites and locations for field nurseries where on-site production of agents could occur. Additionally, the terpenoid concentrations were compared with those from other native, naturalized and ornamental populations. For comparison, these foliar terpenoid profiles were compared with those collected from the species' native Australian population. Analyses are also included from collections of areas where this species is not naturalized but grows as an ornamental (e.g., California, Texas, and Central America). These results will assist in the interpretation of the impact of biological control agents at different release sites where variable host quality occurs.

2. Methods

2.1. Plant collections

Leaf samples were collected from mature *M. quinquenervia* plants from 28 sites in Florida, USA. Collections consisted of one to two fully expanded leaves (~100 mg) placed directly into EtOH (95%) and brought back to the laboratory where they were stored (−10° C) until analysis. Leaf collections were made during the Florida growing season from Apr to Nov 2003. The Florida sites were distributed either along the Atlantic coast (15 sites), the Gulf coast (seven sites) or inland (six sites) from the southern end of the state (Key Largo) to the northern range of the weed. Trees also were sampled north of its naturalized range in Florida from a garden plot in Gainesville, FL where the plants were grown under shade for feeding and testing potential biological control agents in quarantine.

Naturalized populations of *M. quinquenervia* also exist on islands in the Caribbean and Hawaii. In the Caribbean, samples were collected on the islands of Puerto Rico at two sites, and in The Bahamas at three sites. In Puerto Rico the two sites were located at La Tortuguero Lagoon and San Juan Bay Estuary (see Pratt et al., 2005b for site descriptions).

In the Bahamas, *M. quinquenervia* leaf samples were collected at Andros Town, Nassau Airport, and Rocky Creek (see Pratt et al., 2007 for site descriptions). A single population was sampled on the Hawaiian Island Maui near Keanae Point. To characterize the chemotypes of each Florida, Puerto Rico, The Bahamas, and Hawaiian population approximately 100 trees were sampled from each site; though at several sites fewer trees were available. Where the *M. quinquenervia* population was abundant, the sampled trees were separated by at least 10 m.

To compare terpenoid levels in this species' native and naturalized ranges, trees were sampled on a north to south transect along the eastern coast of Australia in 2002 (May to Jun; $N = 78$) and 2004 (Jul; $N = 114$). In Australia generally five to 10 trees were sampled at each location. Sites were also sampled at other locations where this species is not naturalized and is only known from ornamental plantings. These included San Diego, CA (Dec 2002; $N = 5$), La Feria, TX (Feb 2004; $N = 2$), the St. John, U.S. Virgin Islands (Jul 2003; $N = 2$), and San Juan, Costa Rica (Apr 2004; $N = 12$). At these locations all available trees were sampled.

2.2. Gas chromatography

The *M. quinquenervia* foliar terpenoid constituents were determined by solvent extraction using EtOH and dried overnight over Na_2SO_4. After 5 d extraction in solvent, each sample was analyzed by gas chromatography (GC). The results of these solvent extractions were similar to those from microwave extractions (Wheeler, 2006). To quantify the foliar constituents, extracts were analyzed with an Agilent model 6890 GC. Data collection, storage, and analysis were conducted with the Agilent ChemStation (Wilmington, DE) data system. Helium was used as a carrier gas at a linear flow rate of 37 cm/s. All samples were injected (1 μl) with an autosampler (HP-7683) with a 1:50 split on a fused silica capillary column (DB-17 MS Agilent; 30 m × 0.32 mm i.d., 0.25 micron thick film). Both the injector and FID temperatures were 250 °C. The oven temperature was held at 50 °C for 2 min then increased at 8 °C/min to 250 °C where it was held isothermal for 10 min. Constituent quantification was determined by linear regression using external standards of known concentrations. Terpenoid standards were purchased from commercial sources (e.g., Sigma, St Louis, MO, USA) or donated (viridiflorol and 2,4-dihydroxy-6-methoxytoluene) by I.A. Southwell (formerly NSW Agriculture, Wollongbar Agricultural Institute, NSW, Australia) and were of the highest purity available (Wheeler et al., 2002).

To confirm these compound identities GC/MS was performed with an Agilent 6890 instrument fitted with either an HP-5MS (Agilent, 30 m × 0.25 mm, 0.25 micron film thickness) or a DB-17MS (J&W Scientific, 30 m × 0.32 mm, 0.25 micron thick film) FSOT column with helium at 36 or 42 cm/s (HP-5MS and DB-17MS, respectively) as a carrier gas. Injections were conducted with an autosampler (HP-7683) split 1:20 at 250 °C. The mass selective detector (HP 5973) was heated at 250 °C (source) and 150 °C (quad) with transfer line 280 °C and ion source filament voltage of 70 eV. Component identification was made on the basis of mass spectral fragmentation, retention index with *n*-paraffins, comparison with authentic constituents when available, and mass spectral and retention matching with commercial libraries (NIST, Wiley, and Adams).

2.3. Data analysis

To visually summarize *M. quinquenervia* populations with respect to their terpenoid chemistry, principal component analysis (SAS/PC; PROC Factor; method = principal; SAS Institute, 1990) was applied to the correlation matrix of the chromatography results (Jolliffe, 2002). The data were transformed prior to analysis by the column centering method (Hibbert, 1997; Jolliffe, 2002). To assist in interpretation, the components were visualized with a varimax (orthogonal) rotation and the loadings for each component were considered meaningful if their absolute values were greater than 0.4. To determine the number of chemotypes present in our analysis, principal components were retained based upon each variable contributing one unit of variance, a scree graph, and each component accounting for at least 5% of the total variance (Jolliffe, 2002).

To compare the concentrations of terpenoids extracted from plants grown in the different regions, e.g., Florida, the Caribbean, Hawaii, and Australia, the transformed (log +0.1; to standardize variance) total terpenoid concentrations were examined with one-way ANOVA. Where sufficient samples were collected for comparison in two regions (e.g., Florida (28 sites), Caribbean (five sites)), the total terpenoids quantified within each region were analyzed by one-way ANOVA. To determine if latitude within Florida (Ireland et al., 2002) influenced the total amount of terpenoids, a linear regression was performed on the transformed (log +0.1) terpenoid yields across latitudes.

3. Results

3.1. Principal component analysis

The results of *M. quinquenervia* GC analysis resulted in the quantification of 28 terpenoid compounds (Table 1). These 28 constituents were used for subsequent principal component analysis to determine the number of chemotypes in the Florida, Caribbean, Hawaiian and Australian populations. These results indicated the first two components accounted for 82% of the variation and together gave an accurate representation of the data (Fig. 1). Additionally the results of scree test (results not shown) also suggested that only the first two components were meaningful. Only these two components were retained for orthogonal rotation. Interpretation of the rotated coefficients assisted in the distinction in the factor patterns. The first component accounted for 70% of the total variation and contrasts *E*-nerolidol with most of the other variables including α-pinene, α-terpineol, 1,8-cineole, and viridiflorol (Fig. 1). Interpretation of this result suggests that this component contrasts plants that had relatively high concentrations of *E*-nerolidol versus those that lacked this compound (Table 2). After this source of variation is removed, the second component accounted for 12% of the total variation and contrasts *E*-nerolidol, humulene, 2,4-dihydroxy-6-methoxytoluene, linalool, and β-caryophyllene with the variables α-pinene, β-pinene, limonene, 1,8-cineole, and α-terpineol. This contrast may be interpreted as terpenoid variation within the viridiflorol chemotype. We were not able to distinguish additional chemotypes as there appears to be a continuous spread of scores without any discrete clumps or separations (Fig. 1). The remaining components each account for less than 4% of the total variation.

Table 1
Constituents of GC and GC/MS analysis of leaves collected from *M. quinquenervia* plants of two chemotypes collected in Florida, USA

Constituent[a]		Chemotype			
		E-nerolidol		Viridiflorol	
		Mean (μg/mg)	SE	Mean (μg/mg)	SE
1	α-Pinene	0.085	0.005	1.134	0.040
2	β-Pinene	0.009	0.001	0.275	0.008
3	Myrcene	0.004	0.001	0.060	0.002
4	α-Phellandrene	0.003	0.000	0.006	0.000
5	α-Terpinene	0.003	0.000	0.009	0.000
6	Limonene	0.031	0.003	0.547	0.017
7	1,8-Cineole	0.056	0.007	2.526	0.120
8	γ-Terpinene	0.005	0.000	0.102	0.004
9	Benzaldehyde	0.011	0.001	0.018	0.002
10	Linalool	0.024	0.002	0.025	0.001
11	Fenchone	0.001	0.000	0.005	0.000
12	Methyl benzoate	0.026	0.006	0.033	0.009
13	Terpinen-4-ol	0.010	0.001	0.108	0.005
14	α-Terpineol	0.026	0.003	0.908	0.041
15	α-Copaene	0.016	0.001	0.065	0.003
16	α-Gurjunene	0.018	0.001	0.125	0.006
17	β-Caryophyllene	0.310	0.010	0.483	0.015
18	(+)Aromadendrene	0.031	0.002	0.134	0.005
19	α-Humulene	0.084	0.002	0.109	0.003
20	(−)Alloaromadendrene	0.014	0.001	0.177	0.006
21	δ-Cadinene	0.051	0.004	0.132	0.004
22	*E*-nerolidol	5.005	0.169	0.017	0.001
23	Globulol	0.023	0.002	0.118	0.008
24	Viridiflorol	0.109	0.019	5.888	0.212
25	Caryophyllene oxide	0.095	0.006	0.273	0.012
26	β-Eudesmol	0.003	0.000	0.027	0.001
27	α-Bisabolol	0.139	0.004	0.143	0.005
28	2-4-Dihydroxy-6-methoxytoluene	0.302	0.009	0.257	0.008
	Total	6.490	0.197	13.694	0.416

[a] Constituents quantified on the DB-17 MS column following EtOH extraction.

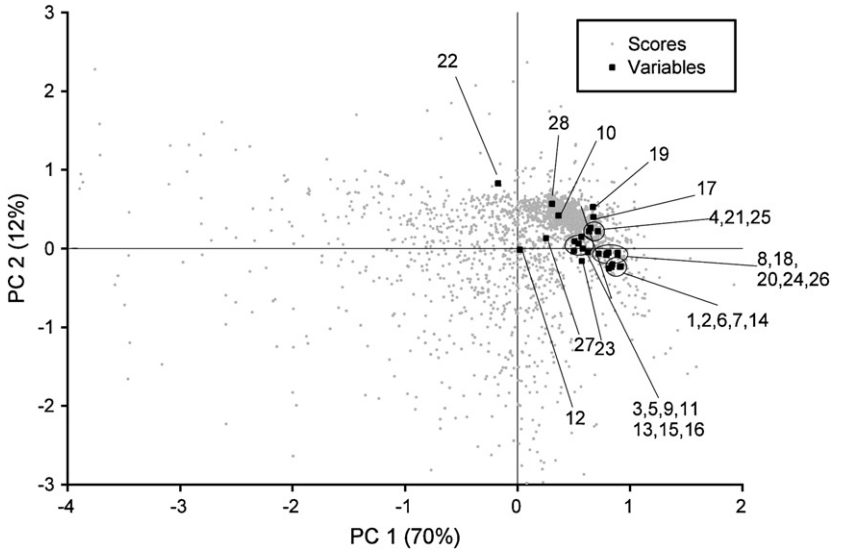

Fig. 1. Principal component analysis of scores calculated from *M. quinquenervia* foliar terpenoid analysis and the variable loadings. Numbers refer to constituents listed in Table 1. Components 1 and 2 explain 70 and 12% of the variation, respectively. The leaf samples were collected from 28 populations in Florida (Table 2).

Table 2
Sites in Florida where *M. quinquenervia* leaves were collected

Map number	Site	GPS coordinates[a]		%Chemotype		Number samples
		Latitude	Longitude	Viridiflorol	*E*-nerolidol	
1	Gainesville Biocontrol Lab	N 29.63501	W 82.37125	98	2	101
2	Lake Conway	N 28.47323	W 81.33632	52	48	100
3	Merritt Island	N 28.52745	W 80.71240	37	63	100
4	Lake Placid	N 27.27000	W 81.36000	98	2	49
5	St. Lucie	N 27.43636	W 80.41194	6	94	82
6	Rim Canal	N 26.78429	W 80.95420	36	64	100
7	Clewiston	N 26.74442	W 80.88866	33	67	100
8	Ponn	N 27.13700	W 80.22798	45	55	99
9	West Palm Beach	N 26.56524	W 80.13238	59	41	101
10	Strazzula Strand	N 26.58511	W 80.23849	44	56	98
11	Markham Park	N 26.14006	W 80.34545	66	34	99
12	Water conservation area	N 26.16227	W 80.36269	98	2	100
13	University and Griffin	N 26.05605	W 80.25168	83	17	100
14	Phase 1 Retention Basin	N 25.77236	W 80.44486	84	16	99
15	South Dade	N 25.81208	W 80.41780	44	56	100
16	Krome Ave.	N 25.71341	W 80.47949	15	85	99
17	Everglades National Park	N 25.60497	W 80.56999	74	26	100
18	Coral Reef Club-Key Largo	N 25.32389	W 80.28487	45	55	11
19	Key Largo	N 25.15686	W 80.38872	41	59	17
20	Belle Meade	N 26.10478	W 81.63392	48	52	99
21	Corkscrew Swamp Sanctuary	N 26.37942	W 81.54566	38	62	100
22	Estero	N 26.42550	W 81.81033	18	82	100
23	Fort Myers	N 26.54698	W 81.79820	44	56	100
24	Prairie Pines	N 26.72668	W 81.87943	24	76	100
25	Manatee County	N 27.48127	W 82.61506	41	59	100
26	Belleview Biltmore	N 27.94183	W 82.80851	33	67	100
27	Windsor Park	N 28.11095	W 82.44820	15	85	100
28	Lake Colebrook	N 28.09852	W 82.46539	29	71	99

[a] Global positioning system in decimal degrees (WGS84 datum).

3.2. Terpenoid chemistry

The major constituents of the *E*-nerolidol chemotype (Table 1) consisted of the terpenoids *E*-nerolidol (5.01 ± 0.17 μg/mg) and β-caryophyllene (0.31 ± 0.01 μg/mg). The viridiflorol chemotype was characterized by the major constituents α-pinene (1.13 ± 0.04 μg/mg), 1,8-cineole (2.53 ± 0.12 μg/mg), α-terpineol (0.91 ± 0.04 μg/mg), β-caryophyllene (0.48 ± 0.01 μg/mg), and viridiflorol (5.89 ± 0.21 μg/mg). The results of the analysis of the total amount of terpenoids extracted indicated that 13.69 (±0.42 μg/mg) were extracted from the viridiflorol compared with 6.49 (±0.20 μg/mg) from the *E*-nerolidol chemotype (Table 1).

3.3. Florida sites

To find populations most conducive to the biological control agents, the proportion of each *M. quinquenervia* chemotype was determined at 28 Florida sites. These proportions were highly variable and did not follow any regional trends (Fig. 2; Table 2). A few sites (e.g., 1, 4, 12, 13, 14, and 17) were comprised predominantly of the viridiflorol chemotype plants whereas other sites were just the opposite; more than 67% of the plants were assigned to the *E*-nerolidol chemotype (e.g., 5, 7, 16, 22, 24, 26, 27, and 28). More than half of the sites (16 of 28), however, had plants that represented a more equal mix of the two chemotypes; between 33 and 67% of the plants were either *E*-nerolidol or viridiflorol chemotype (Fig. 2; Table 2).

3.4. Sites outside Florida

Several locations were sampled outside Florida that included both exotic naturalized and ornamental trees. The locations in Hawaii, Puerto Rico, and the Bahamas, had naturalized populations of *M. quinquenervia* and thus sufficient numbers to characterize chemotype proportions (Table 3). These results indicated that the viridiflorol chemotype predominated at La Tortuguero Lagoon and the majority of the trees sampled at the Hawaii and Nassau sites were the *E*-nerolidol chemotype. Several of these naturalized populations had no clear predominant chemotype (Andros Island,

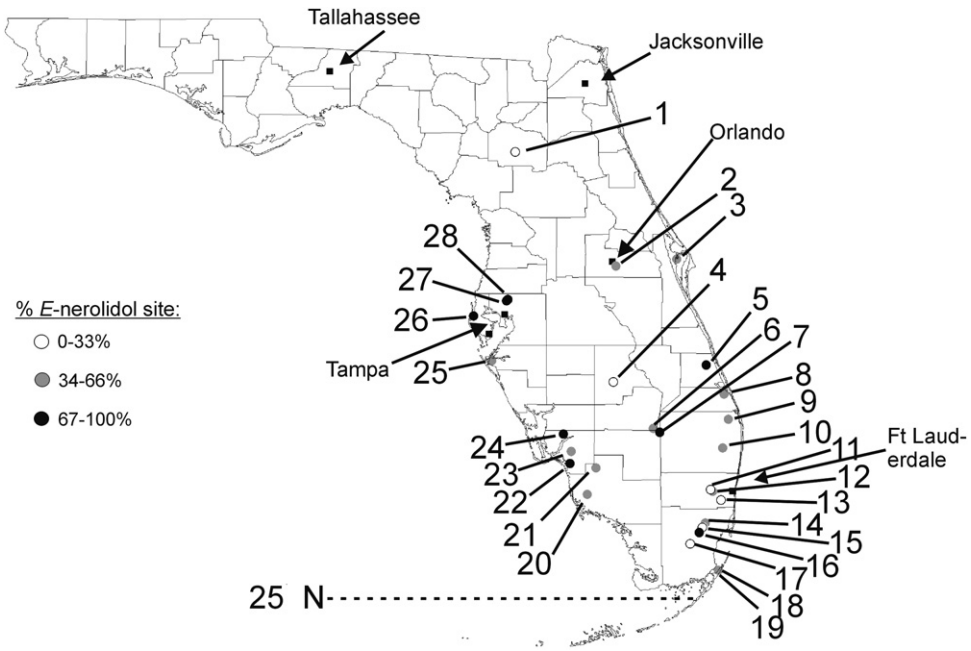

Fig. 2. Distribution of locations in Florida where *M. quinquenervia* foliar samples were collected for terpenoid analysis. Numbers refer to constituents listed in Table 1. Open, gray, and solid dark circles refer to sites where the *E*-nerolidol chemotype constituted 0–33%, 34–66%, and 67–100% of the population, respectively.

Table 3
Sites outside Florida where *M. quinquenervia* populations were collected and the percent of the plants assigned to each chemotype

Region	Location	GPS coordinates[a]		%Chemotype		Number samples
		Latitude	Longitude	Viridiflorol	*E*-nerolidol	
US	Maui, HI	N 20.85895	W 156.16006	4	96	100
	La Feria, TX	N 26.16777	W 97.82383	50	50	2
	Presidio, San Diego, CA	N 32.49188	W 117.15588	0	100	5
Caribbean/Central America	Caneel Bay Resort, St. John, US Virgin Islands	N 18.34460	W 64.78599	100	0	2
	Andros Island, The Bahamas	N 24.71348	W 77.79996	50	50	91
	Rocky Creek, Grand Bahama, The Bahamas	N 26.65899	W 78.00917	44	56	80
	Nassau, The Bahamas	N 25.05827	W 77.45352	5	95	94
	San Juan Bay, Puerto Rico	N 18.42549	W 65.99397	45	55	86
	La Tortuguero Lagoon, Puerto Rico	N 18.47442	W 66.42080	80	20	70
	San Jose, Costa Rica	N 9.56312	W 84.04504	83	17	12

[a] Global positioning system in decimal degrees (WGS84 datum).

The Bahamas; Rocky Creek, The Bahamas; San Juan, Puerto Rico; Table 3). At several sites (Texas, California, The Virgin Islands, and Costa Rica) only ornamental plants were sampled and although fewer than 15 trees were available, a clear chemotype predominated (California; The Virgin Islands; and Costa Rica; Table 3).

Plants were sampled at sites along the eastern coast of the Australian native range of *M. quinquenervia* (Table 4; Fig. 3). The viridiflorol chemotype plant was found most commonly throughout the range sampled in Australia. The *E*-nerolidol plants were found to overlap with the viridiflorol plants at sites between Brisbane and Sydney (Narrabeen). These results confirm those of a previous study (Ireland et al., 2002) and demonstrate the validity of our methods.

3.5. Terpenoid yield comparisons

Populations of *M. quinquenervia* from Australia, Hawaii, Puerto Rico, and the Bahamas were compared in terms of the total amounts of terpenoids recovered with those of Florida. When the total amount from leaves collected from

Table 4
Sites in Australia where *Melaleuca quinquenervia* leaves were collected

Map number	Site	GPS location[a]	
		Latitude	Longitude
1	Laguna Quays	S 20.57183	E 148.62167
2	Sarina	S 21.43783	E 149.26167
3	Keppel Sands	S 23.32900	E 150.78600
4	Agnes Water	S 24.20983	E 151.90650
5	Hervey Bay	S 25.32200	E 152.89367
6	Boonooroo	S 25.66367	E 152.87550
7	Rainbow Beach	S 25.89317	E 153.08150
8	Mudjimba	S 26.60617	E 153.09833
9	Blue Lake, Beach Rd., Stradbroke I.	S 27.49083	E 153.50933
10	Pottsville	S 28.37433	E 153.57217
11	Lennox Heads	S 28.76167	E 153.58550
12	Woodburn	S 29.16950	E 153.28233
13	Arrawarra	S 30.04100	E 153.19000
14	Pt. Macquarie	S 31.43883	E 152.87767
15	Nr. Tuggerah	S 32.01567	E 151.44283
16	Nr. Bulahdelah (Myall L.)	S 32.38467	E 152.39783
17	Bateau Bay	S 33.38333	E 151.47500
18	Tuggerah	S 33.34900	E 151.44283
19	Narrabeen	S 33.69850	E 151.29367

[a] Global positioning system in decimal degrees (WGS84 datum).

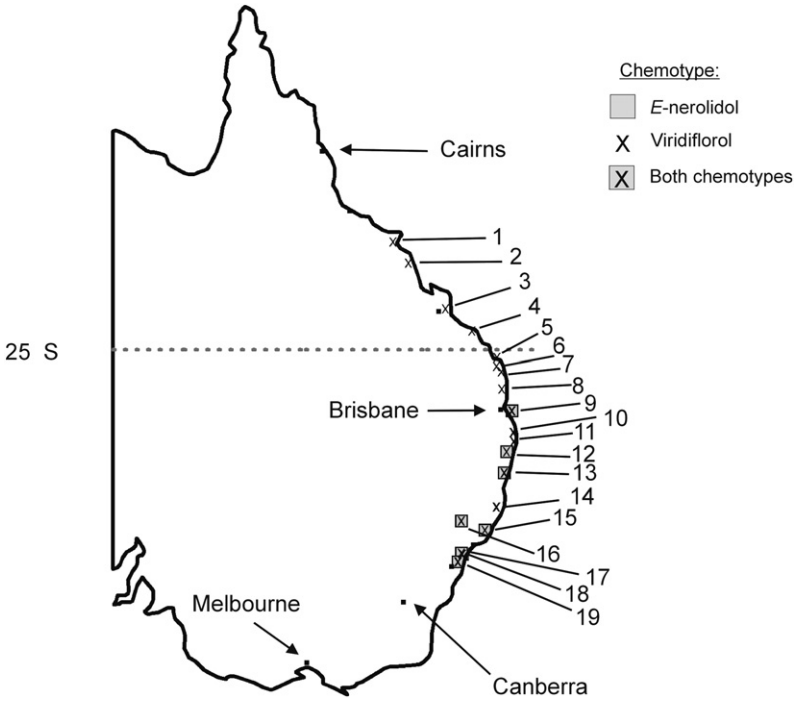

Fig. 3. Distribution of locations in Australia where *M. quinquenervia* foliar samples were collected for terpenoid analysis. Numbers refer to locations listed in Table 4. Locations marked with gray boxes are where only *E*-nerolidol trees were found, 'X' where only viridiflorol trees were found and a combination of the two where both chemotypes were found. None of the sites had only *E*-nerolidol trees.

different regions was compared, both regions ($F = 152.18_{3,2831}$; $P < 0.0001$) and chemotypes ($F = 12.61_{1,2831}$; $P = 0.0004$) and their interaction ($F = 8.78_{3,2831}$; $P < 0.0001$) were significant. Generally, greater total terpenoids were found from plants assigned to the viridiflorol chemotype; with the exception of the *E*-nerolidol plants in Australia (Fig. 4A). Moreover, for both chemotypes the lowest yields were found in plants grown in Hawaii and Florida and the highest were from plants grown in Australia and the Caribbean.

Within Florida, the total amount of terpenoids extracted varied greatly at different sites and was significantly affected by both chemotype ($F = 246.91_{1,2777}$; $P < 0.0001$), site ($F = 113.41_{27,2777}$; $P < 0.0001$) and their interaction ($F = 9.88_{27,2777}$; $P < 0.0001$). At all sites, the total amount quantified from viridiflorol plants was greater than that from the *E*-nerolidol plants. The plants grown at the Gainesville quarantine lab (site 1; Fig. 4B), outside the naturalized range of this species, had the lowest amount of total terpenoids. Similar low levels were recovered from plants grown at Rim canal, Clewiston, and Fort Myers (sites 6, 7, and 23; Fig. 4B). The plants grown at Strazzula strand, Belleview Biltmore, and Windsor Park had the highest amount quantified (sites 10, 26, and 27; Fig. 4B). Within Florida, latitude did not significantly influence yield of either chemotype (both $P > 0.6$).

The total amount quantified from leaves collected at locations at the Caribbean locations was also influenced by chemotype ($F = 94.76_{1,411}$; $P < 0.0001$), site ($F = 79.61_{4,411}$; $P < 0.0001$) and their interaction ($F = 6.52_{4,411}$; $P < 0.0001$). As seen previously for the Florida sites, the viridiflorol plants had greater total amounts than the *E*-nerolidol plants at all sites (Fig. 4C). Generally the greatest amounts were found from plants grown at the Bahamian Andros site and the least amounts were from the Puerto Rico sites San Juan and Tortuguero Lagoon (Fig. 4C).

4. Discussion

The results of principal component analysis of the leaves of *M. quinquenervia* from Florida, the Caribbean, and Hawaii indicated the occurrence of two chemotypes. Similar results are reported for Australia here and by Ireland et al. (2002) where the same two chemotypes were identified, one high in *E*-nerolidol and the second composed of the major constituents 1,8-cineole, viridiflorol, α-terpineol, and β-caryophyllene. Additional chemotypes were not

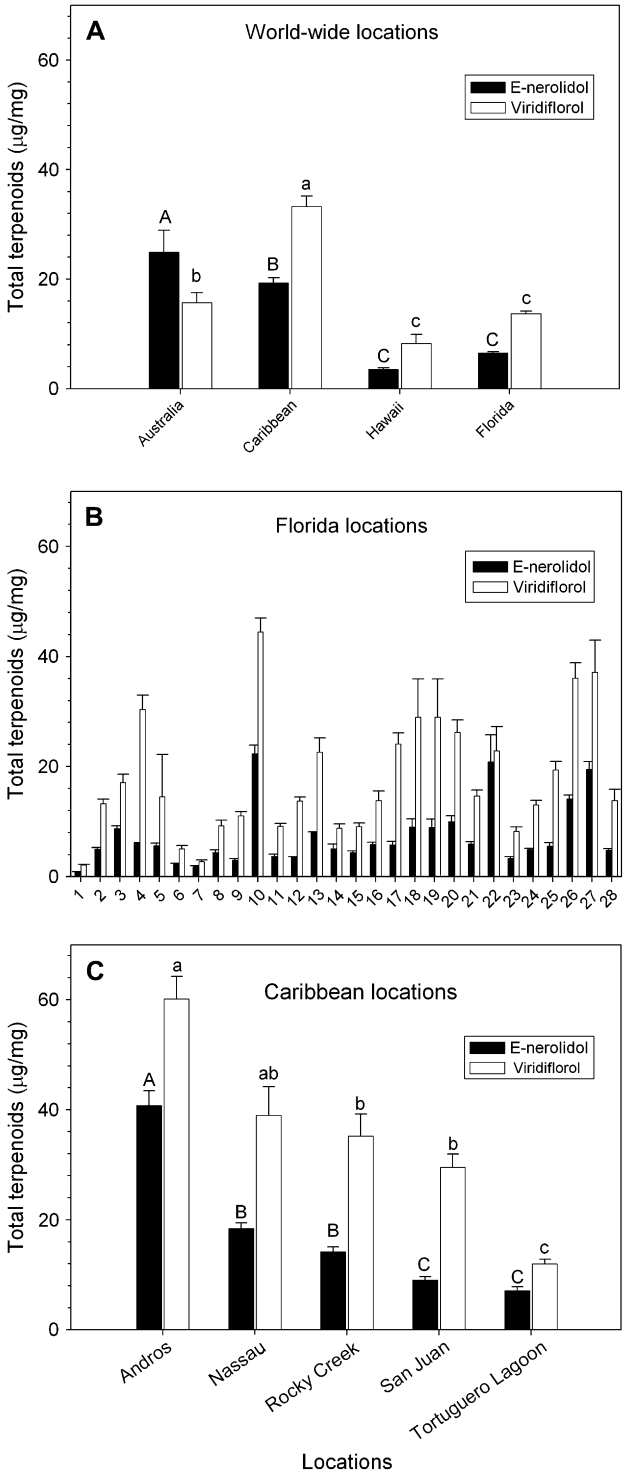

Fig. 4. Mean total amount of terpenoids (μg/mg) quantified from extracts of *M. quinquenervia* foliage. Total terpenoid concentrations from plants assigned to the *E*-nerolidol chemotype in solid bars, those assigned to the viridiflorol chemotype in open bars. Solid bars with the same uppercase letter or open bars with the same lowercase letter are not significantly different according to a Ryan's Q test ($P = 0.05$). Total terpenoids summarized by regions where *M. quinquenervia* is native (Australia) and from areas where the plant is naturalized Caribbean, Hawaii, and Florida (A). Total terpenoids summarized from 28 sites in Florida; see Table 2 for site number descriptions (B). Total terpenoids summarized from Caribbean locations (C) from The Bahamas (Andros, Nassua, and Rocky Creek) and Puerto Rico (San Juan and Tortuguero Lagoon).

apparent from the analysis here as no distinct separation in the scores was visualized and less than 5% of the total variance could be attributed to each of the remaining components. Chemotypes of this species have been selected and cultivated in other regions of the world for various purposes, among them the foliar production of Niaouli essential oils (Ramanoelina et al., 1994; Trilles et al., 2006). For example, in New Caledonia where this species is cultivated for Niaouli, three chemotypes were recognized, however, the *E*-nerolidol chemotype was not among them (Trilles et al., 2006). In Florida, *M. quinquenervia* was introduced and widely planted during the first part of the 1900s (Turner et al., 1998). These plants were subsequently propagated and redistributed by nurserymen as ornamentals (Morton, 1966; Hofstetter, 1991) and for erosion control (Stocker and Sanders, 1981). Unlike other parts of the world, this species was not known to be cultivated in Florida for essential oil production. The origin and history of the Caribbean and Hawaiian populations of *M. quinquenervia* are poorly known. This species was apparently introduced in western Puerto Rico before 1923 as its presence was described in surveys conducted between 1923 and 1926 (Britton and Wilson, 1926). The first published report of *M. quinquenervia* in the Bahamas is in 1978 (Campbell, 1978). Possibly the *M. quinquenervia* chemotypes that exist in southern Florida are a result of artificial selection by nurserymen for ornamental characteristics that are conducive to rapid growth and have commercial value in this area.

The greatest concentrations of *M. quinquenervia* total terpenoids were found from leaves collected in Australia and the Caribbean compared with those from Hawaii and Florida. It is not known, however, whether these differences are due to selection of specific genotypes in these naturalized populations with reduced total terpenoid concentrations or if abiotic factors (e.g., soil fertility and climate), or a combination, produced these differences. Natural selection of *M. quinquenervia* could have occurred in the absence of herbivory in Florida resulting in greater competitive ability and reduced levels of defenses like terpenoids (Blossey and Notzold, 1995). However, studies that assessed the evolution of increased competitive ability (EICA) in uniform garden plots of *M. quinquenervia* genotypes from Florida and Australia did not support this hypothesis (Franks et al., in press). Moreover, the similarly high total terpenoid levels in naturalized *M. quinquenervia* populations in the Caribbean and Australian collections do not support this hypothesis.

Our results indicated that the lowest concentrations of *M. quinquenervia* foliar total terpenoids found in Florida were from plants grown at the Gainesville biological control quarantine facility where potential agents undergo risk assessment studies. Although the seeds for these plants originated from various locations in southern Florida, these results suggest that the plants grown outside their naturalized range may not be representative, in terms of terpenoid levels, of plants targeted for control. To determine the significance of these results, studies need to be conducted that measure the effect of reduced terpenoid levels on herbivore host selection, survival, and performance. Additionally, the majority (98%) of the quarantine *M. quinquenervia* plants were of the viridiflorol chemotype, found in earlier research to be less nutritious for *O. vitiosa* (Wheeler, 2006). These results help to explain difficulty experienced during the early phases of the project when quarantine populations of weevils were fed these plants and failed to reproduce (G.R. Buckingham, USDA/ARS, Gainesville, FL and M.F. Purcell, CSIRO, Brisbane, Australia unpublished data).

The proportion of each chemotype of *M. quinquenervia* at different populations in Florida was highly variable with more than half of the populations composed of about equal numbers of plants assigned to each chemotype. Potential release sites and nursery production sites could be identified in at least eight populations in Florida where the more nutritious *E*-nerolidol chemotype predominated. Among these was the Estero population where the more compatible chemotype dominates the population. In addition to the *E*-nerolidol chemotype leaves, the weevil, *O. vitiosa*, is dependent upon young leaves for larval food (Purcell and Balciunas, 1994; Wheeler, 2001) and a short hydroperiod or generally dry soils for pupation (Purcell and Balciunas, 1994; Center et al., 2000). The Estero population was the most productive nursery site during these initial release and redistribution efforts due to several factors; among them, the regrowth of young leaves from cut stumps (Center et al., 2000). At the time the terpenoid chemistry of the plants and its influence on weevil performance was not known (Wheeler, 2006). However, considering that this site was dominated (82% of the plants) by the more nutritious *E*-nerolidol chemotype, it is now recognized that the terpenoid chemistry of *M. quinquenervia* was an important factor in the establishment of *O. vitiosa*.

Acknowledgments

We are indebted to the technical assistance of S. Wiggers and K. Balentine AmeriCorps, Student Conservation Association, and I.A. Southwell (New South Wales Agriculture, Wollongbar Agricultural Institute, Wollongbar, Australia) who generously provided assistance in terpenoid identification and donations of viridiflorol and 2,

4-dihydroxy-6-methoxytoluene standards. We are grateful for the collections of leaf material made by Dan Clark, National Park Service, Virgin Islands; K. Daehler Univ. of Hawaii; K. Davies, Univ. of Adelaide, Australia; Dana Price, Texas Parks and Wildlife, Austin TX. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. Financial support was provided by the Florida Department of Environmental Protection and USDA/ARS TAME *Melaleuca* Area Wide Project.

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